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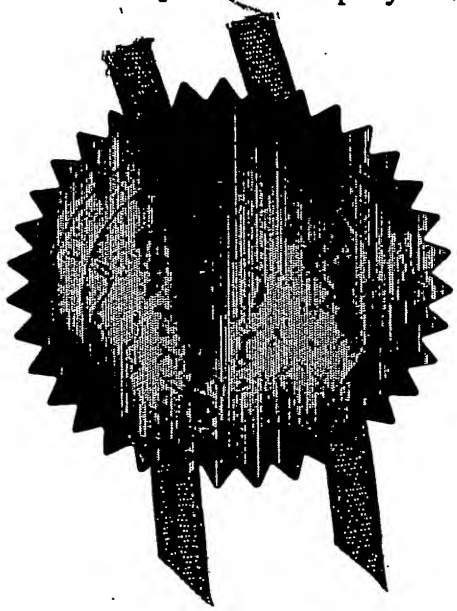

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1. Your reference — P451721

2. Patent application number *(The Patent Office will fill in this part)* — 0227964.4 — 29 NOV 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

RSR Limited
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7094253002

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — England and Wales

4. Title of the invention — Binding Partners for the Thyrotropin Receptor and Uses Thereof

5. Name of your agent *(if you have one)*

Urquhart-Dykes & Lord

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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Number of earlier application	Date of filing *(day / month / year)*

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Binding Partners for the Thyrotropin Receptor and uses thereof

The present invention is concerned with binding partners (such as monoclonal antibodies) for the thyrotropin receptor (TSH receptor or TSHR) and uses thereof.

Thyrotropin or thyroid stimulating hormone (TSH) is a pituitary hormone which plays a key role in regulating the function of the thyroid. Its release is stimulated by the hormone TRH formed in the hypothalamus and TSH controls the formation and release of the important thyroid hormones thyroxine (T4) and tri-iodothyronine (T3). On the basis of a feedback mechanism, the thyroid hormone content of serum controls the release of TSH. The formation of T3 and T4 by thyroid cells is stimulated by TSH by a procedure in which the TSH released by the pituitary binds to the TSH receptor of the thyroid cell membrane.

In Graves' disease (a common autoimmune disorder) TSH receptor autoantibodies (TRAb) are formed and these autoantibodies bind to the TSH receptor in such a way as to mimic the actions of TSH, stimulating the thyroid gland to produce high levels of thyroid hormones. These autoantibodies are described as having stimulating activity. In some patients, autoantibodies bind to the TSH receptor but do not stimulate thyroid hormone production and are described as having blocking activity. (J Sanders, Y Oda, S-A Roberts, M Maruyama, J Furmaniak, B Rees Smith; "Understanding the thyrotrophin receptor function-structure relationship" Ballière's Clinical Endocrinology and Metabolism; Ed TF Davies 1997; **11**(3): 451-479; pub Ballière Tindall, London).

Measurements of TSH receptor autoantibodies are important in the diagnosis and management of Graves' disease and other thyroid disorders. Currently three types of assay are used to measure TSH receptor autoantibodies:-

(a) competitive binding assays which measure the ability of TSH receptor autoantibodies to inhibit the binding of TSH to preparations of TSH receptor;

(b) bioassays which measure the ability of TSH receptor autoantibodies to stimulate cells expressing the TSH receptor in culture; and

(c) immunoprecipitation of TSH receptor preparations with TSH receptor autoantibodies.

Measurement of TSH receptor autoantibodies using such assays are described in references:-

J Sanders, Y Oda, S-A Roberts, M Maruyama, J Furmaniak, B Rees Smith; "Understanding the thyrotrophin receptor function-structure relationship" Ballière's Clinical Endocrinology and Metabolism; Ed TF Davies 1997; **11**(3): 451-479; pub Ballière Tindall, London.

J Sanders, Y Oda, S Roberts, A Kiddie, T Richards, J Bolton, V McGrath, S Walters, D Jaskólski, J Furmaniak, B Rees Smith; "The interaction of TSH receptor autoantibodies with ^{125}I-labelled TSH receptor"; Journal of Clinical Endocrinology and Metabolism 1999; **84**(10): 3797-3802.

It has been recognised for many years that monoclonal autoantibodies to the TSH receptor derived from patients' lymphocytes would be valuable reagents for understanding the pathogenesis of Graves' disease and for developing new methods of measuring TSH receptor autoantibodies (TRAb) for example as replacements for TSH in competitive binding assays. Also, as the patient's serum TSH receptor autoantibodies are usually powerful thyroid stimulators (TSH agonists) stimulating monoclonal TSH receptor autoantibodies would be valuable for in vivo applications when tissue containing the TSH receptor (eg thyroid tissue or thyroid cancer tissue) required stimulation. Furthermore, as some patient serum TSH receptor autoantibodies are powerful TSH antagonists (blocking antibodies) monoclonal TSH receptor autoantibodies which are TSH antagonists would be valuable for in vivo applications when the activity of tissue containing the TSH receptor (eg thyroid tissue or thyroid cancer tissue) required inactivation or to be made unresponsive to TSH, TSH autoantibodies or other stimulators.

It has also been recognised that one of the major advantages of monoclonal TSH receptor autoantibodies over TSH in such in vitro and / or in vivo applications would be the relative ease with which antibodies can be manipulated. For example, manipulation of the TSH receptor binding region of the monoclonal autoantibodies so as to change their characteristics, such as affinity and biological characteristics including their degree of TSH agonist or antagonist activities. Also, monoclonal autoantibodies will have a much longer half life than TSH in vivo and this may have considerable advantages in certain in vivo applications. Furthermore, the half life of antibodies can be manipulated easily, for example antibody Fab fragments have a much shorter half life than intact IgG. These general properties of TSH receptor autoantibodies are described in the publications such as B Rees Smith, SM McLachlan, J Furmaniak; "Autoantibodies to the thyrotropin receptor"; Endocrine Reviews 1988; **9**: 106-121; B Rees Smith, KJ Dorrington, DS Munro; "The thyroid stimulating properties of long-acting thyroid stimulator γG-globulin subunits"; Biochimica et Biophysica Acta 1969; **192**: 277-285; KJ Dorrington, DS Munro; "The long acting thyroid stimulator"; Clinical Pharmacology and Therapeutics 1966; **7**: 788-806.

A still further advantage of monoclonal TSH receptor autoantibodies could be in their use to identify and provide new types of TSH receptor autoantibody binding sites. For example by the generation of antibodies to the regions of the monoclonal TSH receptor autoantibodies which bind the TSH receptor. Some of the anti-idiotypic antibodies produced in this way could have potential as new ligands for assays of TSH receptor autoantibodies, TSH and related compounds. Also they may be effective agents in vivo for regulating the action of TSH receptor autoantibodies, TSH and related compounds.

Other methods of identifying and providing new types of autoantibody binding sites using monoclonal antibodies are well known. For example by antibody screening of phage-displayed random peptide libraries as described by JC Scott and GP Smith; "Searching for peptide ligands with an epitope library"; Science 1990; **249**(4967): 386-390 and MA Myers, JM Davies, JC Tong, J Whisstock, M Scealy, IR MacKay, MJ Rowley; "Conformational epitopes on the diabetes autoantigen GAD_{65} identified

by peptide phage display and molecular modelling"; Journal of Immunology 2000; 165: 3830-3838. Antibody screening of non-peptide compounds and libraries of non-peptide compounds can also be carried out.

New types of TSH receptor autoantibody binding sites identified and provided using these procedures may also be useful as new ligands in assays for TSH receptor autoantibodies, TSH and related compounds. Furthermore they may be effective agents in vivo for regulating the action of TSH receptor autoantibodies, TSH and related compounds.

In view of the potential value of monoclonal TSH receptor autoantibodies there have been considerable efforts over many years to produce such autoantibodies (see for example B Rees Smith, SM McLachlan, J Furmaniak; "Autoantibodies to the thyrotropin receptor"; Endocrine Reviews 1988; **9**: 106-121. However, to date these efforts have been unsuccessful (see for example SM McLachlan, B Rapoport; "Monoclonal, human autoantibodies to the TSH receptor – The Holy Grail and why are we looking for it"; Journal of Clinical Endocrinology and Metabolism 1996; **81**: 3152-3154 and JHW van der Heijden, TWA de Bruin, KAFM Gludemans, J de Kruif, JP Banga, T Logtenberg; "Limitations of the semisynthetic library approach for obtaining human monoclonal autoantibodies to the thyrotropin receptor of Graves' disease"; Clinical and Experimental Immunology 1999; **118**: 205-212).

It is an object of the present invention to provide a binding partner for the TSH receptor capable of interacting with the TSH receptor in a manner comparable to the interaction of TSH receptor autoantibodies with the TSH receptor, in particular it is an object of the present invention to provide human monoclonal autoantibodies to the TSH receptor exhibiting a comparable interaction therewith as seen with TSH receptor autoantibodies present in the sera of patients with hyperthyroid Graves' disease. The considerable difficulties of producing monoclonal TSH receptor autoantibodies have been overcome in the invention described herein. In particular the successful production of a human monoclonal TSH receptor autoantibody with the characteristics of the autoantibodies found in the sera of patients with hyperthyroid Graves' disease is described. The TSH receptor monoclonal autoantibody we have produced (described herein as hMAb TSHR 1) binds to the TSH receptor with high

affinity and in such a way that small amounts of the antibody inhibit labelled TSH binding to the TSH receptor and small amounts act as powerful thyroid stimulators. Fab fragments of the antibody are similarly effective thyroid stimulators and inhibitors of labelled TSH binding as intact IgG. Both monoclonal Fab and intact IgG can be labelled with ^{125}I and shown to bind to the TSH receptor. Such binding is inhibited by TSH receptor autoantibodies in patient sera.

There is provided by the present invention, therefore, a binding partner for the TSH receptor, which binding partner comprises, or is derived from, a human monoclonal or recombinant autoantibody, or one or more fragments thereof, reactive with the TSH receptor.

In particular, there is provided by the present invention a binding partner for the TSH receptor, which binding partner comprises, or is derived from, a human monoclonal autoantibody, or one or more fragments thereof, reactive with the TSH receptor.

In particular, there is provided by the present invention a human monoclonal autoantibody, or one or more fragments thereof, reactive with the TSH receptor.

A binding partner according to the present invention, and in particular, a human monoclonal or recombinant autoantibody reactive with the TSH receptor according to the present invention can be further characterised by its ability to inhibit TSH binding to the TSH receptor, and / or its ability to stimulate the TSH receptor, both of which have been seen to be comparable to the respective inhibitory and stimulatory properties of TSH receptor autoantibodies present in sera obtained from patients with Graves' disease.

More particularly, a binding partner according to the present invention, and in particular a human monoclonal or recombinant autoantibody according to the present invention, can be characterised by an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of

International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 140 units of International Standard NIBSC 90/672 per mg, or one or more fragments of such human or recombinant autoantibody.

More particularly, a binding partner according to the present invention, and in particular a human monoclonal or recombinant autoantibody according to the present invention, can be further characterised by a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 170 units of International Standard NIBSC 90/672 per mg, or one or more fragments of such human or recombinant autoantibody.

In a preferred embodiment of the present invention, a binding partner according to the present invention, and in particular a human monoclonal or recombinant autoantibody according to the present invention, can be characterised by:

(i) an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 140 units of International Standard NIBSC 90/672 per mg; and

(ii) a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 170 units of International Standard NIBSC 90/672 per mg;

or one or more fragments of such human or recombinant autoantibody.

In the case where a binding partner according to the present invention comprises or is derived from one or more fragments of a monoclonal or recombinant autoantibody reactive with the TSH receptor, in particular for example one of more Fab fragments of a monoclonal or recombinant autoantibody reactive with the TSH receptor, it may be preferred that such a binding partner can be characterised by an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 100 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 200 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 250 units of International Standard NIBSC 90/672 per mg, and more preferably of at least about 280 units of International Standard NIBSC 90/672 per mg.

It may also be preferred in the case where a binding partner according to the present invention comprises or is derived from one or more fragments of a monoclonal or recombinant autoantibody reactive with the TSH receptor, in particular for example one of more Fab fragments of a monoclonal or recombinant autoantibody reactive with the TSH receptor, that such a binding partner can be characterised by a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 100 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 200 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 300 units of International Standard

NIBSC 90/672 per mg, or more preferably of at least about 450 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 600 units of International Standard NIBSC 90/672 per mg, or even more preferably of at least about 690 units of International Standard NIBSC 90/672 per mg.

It may be still further preferred in the case where a binding partner according to the present invention comprises or is derived from one or more fragments of a monoclonal or recombinant autoantibody reactive with the TSH receptor, in particular for example one of more Fab fragments of a monoclonal or recombinant autoantibody reactive with the TSH receptor, that such a binding partner can be characterised by:

(i) an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 100 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 200 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 250 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 280 units of International Standard NIBSC 90/672 per mg; and

(ii) a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 100 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 200 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 300 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 450 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 600 units of International Standard NIBSC 90/672 per mg, or even more preferably of at least about 690 units of International Standard NIBSC 90/672 per mg.

In a preferred case the present invention provides a binding partner for the TSH receptor (typically a human monoclonal autoantibody), which binding partner is capable of binding to the TSH receptor so as to stimulate the TSH receptor and which comprises an antibody V_H domain selected from the group consisting of a V_H domain as shown in SEQ ID NO. 1 and a V_H domain comprising one or more V_H CDRs with

an amino acid sequence selected from SEQ ID NO. 2, SEQ ID NO. 3 and SEQ ID NO. 4.

In a first embodiment of the present invention, there is, therefore, provided a binding partner for the TSH receptor (typically a human monoclonal autoantibody), which binding partner is capable of binding to the TSH receptor so as to stimulate the TSH receptor and which comprises an antibody V_H domain as shown in SEQ ID NO. 1.

In a second embodiment of the present invention there is, therefore, provided a binding partner for the TSH receptor (typically a human monoclonal autoantibody), which binding partner is capable of binding to the TSH receptor so as to stimulate the TSH receptor and which comprises an antibody V_H domain comprising one or more V_H CDRs with an amino acid sequence selected from SEQ ID NO. 2, SEQ ID NO. 3 and SEQ ID NO. 4.

It will be appreciated that a binding partner according to the present invention can comprise an antibody V_H domain substantially as hereinbefore described in the absence of an antibody V_L domain. It is known that single immunoglobulin domains, especially V_H domains, are capable of binding target antigens in a specific manner. Alternatively, a binding partner according to the present invention can comprise an antibody V_H domain paired with an antibody V_L domain to provide an antibody binding site comprising both V_H and V_L domains for a TSH receptor employing techniques well known in the art (Biochim. Biophys. Acta, 192 (1969) 277-285; Proc. Natl. Acad. Sci. USA, Vol. 89, pp 10026-10030, November 1992).

One or more CDRs as referred to above may be taken from the hereinbefore described V_H domain and incorporated into a suitable framework. For example, the amino acid sequence of one or more CDRs substantially as hereinbefore described may be incorporated into framework regions of antibodies differing from hMAb TSHR 1 specifically disclosed herein, such antibodies thereby incorporating the one or more CDRs and being capable of binding to the TSH receptor, preferably to stimulate the TSH receptor substantially as hereinbefore described. Alternatively, the present invention may provide a polypeptide capable of binding to the TSH receptor so as to stimulate the TSH receptor substantially as hereinbefore described and comprising the

primary structural conformation of amino acids as represented by one or more CDRs as specifically described herein, optionally together with further amino acids, which further amino acids may enhance the binding affinity of one or more CDRs as described herein for the TSH receptor or may have substantially no role in affecting the binding properties of the polypeptide for the TSH receptor.

The present invention, also encompasses variants, analogs, derivatives and fragments of the specific human monoclonal autoantibody described herein, V_H domains, CDRs and polypeptides disclosed herein, which variants, analogs, derivatives and fragments retain the ability to interact with the TSH receptor (such as for example to stimulate the TSH receptor) substantially as hereinbefore described.

The terms "variants", "analogs", "derivatives" and "fragments" as used herein can be characterised as antibodies, antibody fragments or polypeptides which retain essentially the same biological function or activity as a human monoclonal autoantibody having a V_H domain as shown in SEQ ID NO:1 and in particular in respect of the binding properties thereof for the TSH receptor. Suitably, variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments as described herein, have a primary structural conformation of amino acids in which several or a few (such as 5 to 10, 1 to 5 or 1 to 3) amino acid residues of a human monoclonal autoantibody having a V_H domain as shown in SEQ ID NO:1 are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions are deletions which do not alter or substantially alter the biological activity or function of a human monoclonal autoantibody having a V_H domain as shown in SEQ ID NO:1. Conservative substitutions can be preferred as hereinafter described in greater detail.

More particularly, variants, analogs or derivatives of a human monoclonal autoantibody having a V_H domain as shown in SEQ ID NO:1 according to the present invention may be ones in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), or ones in which one or more of the amino acid resides includes a substituent group or the like. Such variants, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Most typically, variants, analogs or derivatives are those that vary from a reference human monoclonal autoantibody having a V_H domain as shown in SEQ ID NO:1 by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids A, V, L and I; among the hydroxyl residues S and T; among the acidic residues D and E; among the amide residues N and Q; among the basic residues K and R; and among the aromatic residues F and Y.

It will be appreciated that the term fragment as used herein in particular relates to fragments of antibodies specifically as herein described and form an important aspect of the present invention. In this way, a human monoclonal or recombinant autoantibody as provided by the present invention may be provided as any of the following fragments: (i) the Fab fragment consisting of V_L, V_H, C_L and C_H1 domains; (ii) the Fd fragment consisting of the V_H and C_H1 domains; (iii) the Fv fragment consisting of the V_L and V_H domains; (iv) the dAb fragment which consists of a V_H domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments; and (vii) single chain Fv molecules (scFv), wherein a V_H domain and a V_L domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site.

Alternatively, a human monoclonal or recombinant autoantibody according to the present invention may compris- a whole IgG antibody, whereby the antibody includes variable and constant regions, which variable and constant regions can be further illustrated for the antibodies provided by the present invention by reference to SEQ ID NO. 5.

The present invention also provides a further binding partner capable of binding to the TSH receptor so as to stimulate the TSH receptor substantially as hereinbefore described, and which further binding partner can compete for binding to the TSH receptor with a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described, which further binding partner does not comprise TSH or autoantibodies to the TSH receptor. In particular this

further binding partner may comprise a further antibody having a binding site for an epitope region of the TSH receptor, which further antibody is capable of binding to the TSH receptor so as to stimulate the TSH receptor substantially as hereinbefore described and can compete for binding to the TSH receptor with a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described.

Suitably such a further binding partner can be derived from a specific binding partner as described herein, hMAb TSHR 1, by suitable mutagenesis techniques, such as spot mutations or the like, so as to obtain a further binding partner for the TSH receptor that can compete with a binding partner substantially as herein described (such as hMAb TSHR 1) for interaction with the TSH receptor.

Preferably such a further binding partner for the TSH receptor can comprise a monoclonal or recombinant antibody and can be characterised by an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 140 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg, or one or more fragments of the antibody. It may also be preferred that such a further binding partner according to the present invention, can be further characterised by a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 170 units of International Standard NIBSC 90/672 per mg, or one or more fragment of the antibody.

It may also be even more preferred that such a further binding partner of the present invention, can be characterised by:

(i) an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 140 units of International Standard NIBSC 90/672 per mg; and

(ii) a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 170 units of International Standard NIBSC 90/672 per mg;

or one or more fragments thereof.

There is also provided by the present invention a polynucleotide comprising:

(i) a nucleotide sequence as shown in SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8 or SEQ ID NO. 9, encoding an amino acid sequence of an antibody V_H domain, or V_H CDR as shown in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3 or SEQ ID NO. 4;

(ii) a nucleotide sequence encoding a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described, or encoding an amino acid sequence of an antibody V_H domain, or V_H CDR of a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described;

(iii) a nucleotide sequence differing from any sequence of (i) in codon sequence due to the degeneracy of the genetic code;

(iv) a nucleotide sequence comprising an allelic variation of any sequence of (i);

(v) a nucleotide sequence comprising a fragment of any of the sequences of (i), (ii), (iii), or (iv) and in particular a nucleotide sequence comprising a fragment of any of the sequences of (i), (ii), (iii), (iv) or (v) and encoding a Fab fragment, a Fd fragment, a Fv fragment, a dAb fragment, an isolated CDR region, F(ab')2 fragments or a scFv fragment, of a human monoclonal autoantibody substantially as hereinbefore described;

(vi) a nucleotide sequence differing from the any sequence of (i) due to mutation, deletion or substitution of a nucleotide base and encoding a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described, or encoding an amino acid sequence of an antibody V_H domain, or V_H CDR of a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described.

Variant polynucleotides according to the present invention are suitably at least 70% identical over their entire length to any polynucleotide sequence of (i), most highly preferred are polynucleotides that comprise a region that is at least 80% identical over its entire length to any polynucleotide sequence of (i), polynucleotides at least 90% identical over their entire length to any polynucleotide sequence of (i) are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% identity are especially preferred.

The present invention further provides a biologically functional vector system which carries a polynucleotide substantially as hereinbefore described and which is capable of introducing the polynucleotide into the genome of a host organism.

The present invention also relates to host cells which are transformed with polynucleotides of the invention and the production of binding partners for the TSH receptor (typically human monoclonal autoantibodies) of the invention by recombinant techniques. Host cells can be genetically engineered to incorporate polynucleotides and express binding partners for the TSH receptor (typically human monoclonal autoantibodies) of the present invention.

The amino acid sequences of hMAb TSHR 1, a human monoclonal autoantibody according to the present invention, and nucleotide sequences coding therefor, are shown in the Sequence listings as herein after described and can be assigned as follows:

Amino Acid Sequences

SEQ 1D NO. 1	V_H
SEQ ID NO. 2	V_H CDRI
SEQ ID NO. 3	V_H CDRII
SEQ ID NO. 4	V_H CDRIII
SEQ ID NO. 5	Heavy chain variable and constant regions

Nucleotide Sequences

SEQ ID NO. 6	V_H
SEQ ID NO. 7	V_H CDRI
SEQ ID NO. 8	V_H CDRII
SEQ ID NO. 9	V_H CDRIII.
SEQ ID NO. 10	Heavy chain variable and constant regions.

The above sequences for TSHR1 can also be seen by reference to Figures 4 and 5.

The present invention also provides a process of providing a human monoclonal autoantibody to the TSH receptor substantially as hereinbefore described, which process comprises:

(i) providing a source of lymphocytes from a subject, which subject has TSH receptor autoantibody activity of greater than about 0.1 units of NIBSC 90/672 per mL with respect to inhibition of TSH binding to the TSH receptor;

(ii) isolating lymphocytes from said lymphocyte source of (i);

(iii) immortalising the isolated lymphocytes; and

(iv) cloning the immortalised lymphocytes so as to produce an immortalised colony secreting a human monoclonal autoantibody to the TSH receptor substantially as hereinbefore described.

Alternatively, a process of providing a human monoclonal autoantibody to the TSH receptor substantially as hereinbefore described can be defined as a process which comprises:

(i) providing a source of lymphocytes from a subject, which subject has TSH receptor autoantibody activity of greater than about 0.5 units of NIBSC 90/672 per mL with respect to stimulatory activity of cAMP production by cells expressing the TSH receptor;

(ii) isolating lymphocytes from said lymphocyte source of (i);

(iii) immortalising the isolated lymphocytes; and

(iv) cloning the immortalised lymphocytes so as to produce an immortalised colony secreting a human monoclonal autoantibody to the TSH receptor substantially as hereinbefore described.

Preferably a process according to the present invention comprises isolating lymphocytes from peripheral blood, thyroid tissue, spleen tissue, lymph nodes or bone marrow, most typically from peripheral blood. Typically, the source of lymphocytes for use in a method according to the present invention can be further characterised as being obtained from a subject having TSH receptor autoantibody levels of greater than about 0.2 units of NIBSC 90/672 per mL with respect to inhibition of TSH binding to the TSH receptor, or more typically greater than about 0.25 units of NIBSC 90/672 per mL with respect to inhibition of TSH binding to the TSH receptor, or more typically greater than about 0.3 units of NIBSC 90/672 per mL with respect to inhibition of TSH binding to the TSH receptor and preferably being in the range of about 0.3 to 0.5 units of NIBSC 90/672 per mL or greater with respect to inhibition of TSH binding to the TSH receptor. Alternatively, or additionally, the source of lymphocytes for use in a method according to the present invention can typically be further characterised as being obtained from a subject having TSH receptor autoantibody levels of greater than about 0.5 units of NIBSC 90/672 per mL with respect to stimulatory activity of cAMP production by cells expressing the TSH receptor, or more typically greater than about 1.0 units of NIBSC 90/672 per mL with respect to stimulatory activity of cAMP production by cells expressing the TSH receptor and preferably being in the range of about 1.0 to 2.0 units of NIBSC 90/672 per mL or greater with respect to stimulatory activity of cAMP production by cells expressing the TSH receptor. It will be appreciated from the above that the immune response to the TSH receptor of a subject from which lymphocytes are isolated should preferably be in a highly active phase.

Preferably a process according to the present invention comprises infecting the isolated lymphocytes with Epstein Barr virus, and suitably the thus immortalised lymphocytes are fused with a mouse / human cell line. Suitably a process according to the present invention further comprises screening the resulting clones for TSH receptor autoantibodies, for example by inhibition of ^{125}I TSH binding to the TSH receptor in an assay system which has a sensitivity of at least about 1unit/L of NIBSC 90/672.

The present invention further provides a human monoclonal autoantibody to the TSH receptor obtained by a process substantially as described above. Preferably such an

obtained human monoclonal autoantibody to the TSH receptor according to the present invention, can be characterised by an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 140 units of International Standard NIBSC 90/672 per mg, or one or more fragments of such a human monoclonal autoantibody.

More particularly, it may be preferred that such a human monoclonal autoantibody according to the present invention, can be further characterised by a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 170 units of International Standard NIBSC 90/672 per mg, or one or more fragments of such a human monoclonal autoantibody.

In a preferred embodiment of the present invention, such a human monoclonal autoantibody according to the present invention, can be characterised by:

(i) an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg and even

more preferably of at least about 140 units of International Standard NIBSC 90/672 per mg; and

(ii) a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 170 units of International Standard NIBSC 90/672 per mg;

or one or more fragments of such a human monoclonal autoantibody.

It may also be preferred that one or more fragments of thus obtained human monoclonal autoantibody according to the present invention, in particular for example one of more Fab fragments thereof, can be characterised by an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 100 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 200 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 250 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 280 units of International Standard NIBSC 90/672 per mg. It may also be preferred that such one or more fragments can be characterised by a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 100 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 200 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 300 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 450 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 600 units of International Standard NIBSC 90/672 per mg, or even more preferably of at least about 690 units of International Standard NIBSC 90/672 per mg.

More preferably, such one or more Fab fragments can be characterised by:

(i) an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 100 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 200 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 250 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 280 units of International Standard NIBSC 90/672 per mg; and

(ii) a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 100 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 200 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 300 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 450 units of International Standard NIBSC 90/672 per mg, or more preferably of at least about 600 units of International Standard NIBSC 90/672 per mg, or even more preferably of at least about 690 units of International Standard NIBSC 90/672 per mg.

A process substantially as described above may further comprise a further process stage whereby the obtained human monoclonal autoantibody is subjected to suitable further processing techniques (such as suitable mutagenesis techniques, such as spot mutations or the like), so as to obtain a further binding partner for the TSH receptor that can compete with a binding partner substantially as herein described (such as hMAb TSHR 1) for interaction with the TSH receptor. Such further processing techniques are well known to one of ordinary skill in the art. The present invention further provides a further binding partner to the TSH receptor obtained by such further processing techniques.

Preferably such a further binding partner for the TSH receptor can comprise a monoclonal or recombinant antibody and can be characterised by an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 50 units of

International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 140 units of International Standard NIBSC 90/672 per mg, or one or more fragments thereof. It may also be preferred that such a further binding partner according to the present invention, can be further characterised by a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg, of at least about 150 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 170 units of International Standard NIBSC 90/672 per mg, or one or more fragments thereof.

It may also be even more preferred that such a further binding partner of the present invention, can be characterised by:

(i) an inhibitory activity with respect to TSH binding to the TSH receptor, of at least about 50 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 140 units of International Standard NIBSC 90/672 per mg; and

(ii) a stimulatory activity with respect to cAMP production by cells expressing the TSH receptor, of at least about 50 units of International Standard NIBSC

90/672 per mg, more preferably of at least about 70 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 90 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 110 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 130 units of International Standard NIBSC 90/672 per mg, more preferably of at least about 150 units of International Standard NIBSC 90/672 per mg and even more preferably of at least about 170 units of International Standard NIBSC 90/672 per mg;

or one or more fragments thereof.

A binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention may have diagnostic and therapeutic applications.

Accordingly, a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention can be employed in screening methods for detecting autoantibodies to the TSH receptor in patient sera and also in diagnostic methods. In this way, a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention can be employed in place of, or in addition to, competitors hitherto described for use in screening methods for detecting autoantibodies to the TSH receptor and also in diagnostic methods. Similarly, a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention can be employed in place of, or in addition to, competitors hitherto described for use in kits for use in detecting autoantibodies to the TSH receptor.

The present invention also provides, therefore, a method of screening for autoantibodies to the TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing said sample of body fluid from said subject;

(b) providing one or more pairs of binding molecules, wherein a first molecule of said binding pair comprises a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention and a second molecule of said binding pair comprises a binding region with which said binding partner interacts;

(c) contacting said sample with said one or more pairs of binding molecules so as to permit said second molecule of said binding pair to interact with either (i) autoantibodies to the TSH receptor present in said sample, or (ii) said binding partner for the TSH receptor (typically a human monoclonal autoantibody); and

(d) monitoring the interaction of said second molecule of said binding pair with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to the TSH receptor in said sample.

It will be appreciated that binding molecules of the one or more binding pairs can be antigen-antibody (for example, [TSH receptor or epitope]–[monoclonal TSH receptor autoantitibody]), anti-idiotypic antibody-monoclonal TSH receptor autoantitibody or novel TSH receptor autoantitibody binding member-monoclonal TSH receptor autoantitibody. Preferably, the binding molecules of the binding pairs are antigen-antibody, namely, [TSH receptor or one or more epitopes thereof]–[monoclonal TSH receptor autoantitibody, where the epitopes may be "free standing" or present in a larger scaffold polypeptide.

Preferably, the present invention provides, therefore, a method of screening for autoantibodies to the TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing said sample of body fluid from said subject;

(b) contacting said sample with (i) a full length TSH receptor, or one or more epitopes thereof or a polypeptide comprising one or more epitopes of a TSH receptor, and (ii) a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention, under conditions that allow interaction of the TSH receptor with autoantibodies produced in response to the TSH receptor, so as to permit said TSH receptor, or said one or more epitopes thereof or said polypeptide, to interact with either autoantibodies to the TSH receptor present in said sample, or said binding partner for the TSH receptor (typically a human monoclonal autoantibody); and

(c) monitoring the interaction of said TSH receptor, or said one or more epitopes thereof or said polypeptide, with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to the TSH receptor in said sample.

In certain embodiments, a method according to the present invention may also employ one or more competitors that can compete in the interaction of the binding partner of the present invention and the second molecule of the binding pair, such as the TSH receptor or epitopes thereof substantially as hereinbefore described. Such competitors may comprise TSH, or one or more monoclonals reactive with the TSH receptor, such as mouse monoclonals reactive with the TSH receptor.

Preferably, a method according to the present invention as referred to above, further comprises providing labelling means for a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention and where appropriate one or more competitors as described above, suitable labelling means including enzymic labels, isotopic labels, chemiluminescent labels, fluorescent labels, dyes and the like.

The present invention also provides, a kit for screening for autoantibodies to the TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said kit comprising:

(a) one or more pairs of binding molecules, wherein a first molecule of said binding pair comprises a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention and a second molecule of said binding pair comprises a binding region with which said binding partner interacts;

(b) means for contacting said sample of body fluid from said subject with said one or more pairs of binding molecules so as to permit said second molecule of said binding pair to interact with either (i) autoantibodies to the TSH receptor present in said sample, or (ii) said binding partner for the TSH receptor (typically a human monoclonal autoantibody); and

(c) means for monitoring the interaction of said second molecule of said binding pair with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to the TSH receptor in said sample.

It will be appreciated that binding molecules of the one or more binding pairs can be antigen-antibody (for example, [TSH receptor or epitope]–[monoclonal TSH receptor autoantitibody]), anti-idiotypic antibody-monoclonal TSH receptor autoantitibody or novel TSH receptor autoantitibody binding member-monoclonal TSH receptor autoantitibody. Preferably, the binding molecules of the binding pairs are antigen-antibody, namely, [TSH receptor or one or more epitopes thereof]–[monoclonal TSH receptor autoantitibody, where the epitopes may be "free standing" or present in a larger scaffold polypeptide.

The present invention preferably provides a kit for screening for autoantibodies to the TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to the TSH receptor, said kit comprising:

(a) a full length TSH receptor, or one or more epitopes thereof or a polypeptide comprising one or more epitopes of the TSH receptor;

(b) a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention;

(c) means for contacting said sample of body fluid from said subject, said TSH receptor, or said one or more epitopes thereof or said polypeptide, and said binding partner for the TSH receptor (typically a human monoclonal autoantibody), under conditions that allow interaction of the TSH receptor with autoantibodies produced in response to the TSH receptor, so as to permit said TSH receptor, or said one or more epitopes thereof or said polypeptide, to interact with either autoantibodies to a TSH receptor present in said sample, or said binding partner for the TSH receptor (typically a human monoclonal autoantibody); and

(d) means for monitoring the interaction of said TSH receptor, or said one or more epitopes thereof or said polypeptide, with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to the TSH receptor in said sample.

In certain embodiments, a kit according to the present invention may further comprise one or more competitors that can compete in the interaction of the binding partner of the present invention and the second molecule of the binding pair, such as the TSH receptor or epitopes thereof substantially as hereinbefore described. Such competitors may comprise TSH, or one or more monoclonals reactive with the TSH receptor, such as mouse monoclonals reactive with the TSH receptor.

Suitably, a kit as referred to above further comprises labelling means for a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention and where appropriate one or more competitors as described above, suitable labelling means being substantially as hereinbefore described.

In the presence of autoantibodies to the TSH receptor, binding of the TSH receptor to a binding partner for the TSH receptor (typically a human monoclonal autoantibody) in a method or kit as described above will be decreased.

A binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention can also be employed in assay methods and kits substantially as described above for TSH and related ligands.

The present invention also provides, therefore, a method of assaying TSH and related ligands, said method comprising:

(a) providing a sample suspected of containing or containing TSH or related ligands;

(b) providing one or more pairs of binding molecules, wherein a first molecule of said binding pair comprises a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention and a second molecule of said binding pair comprises a binding region with which said binding partner interacts;

(c) contacting said sample with said one or more pairs of binding molecules so as to permit said second molecule of said binding pair to interact with either (i) TSH or related ligands present in said sample, or (ii) said binding partner for the TSH receptor (typically a human monoclonal autoantibody); and

(d) monitoring the interaction of said second molecule of said binding pair with TSH or related ligands present in said sample, thereby providing an indication of the presence of TSH or related ligands in said sample.

The present invention also provides a kit for assaying TSH or related ligands, said kit comprising:

(a) one or more pairs of binding molecules, wherein a first molecule of said binding pair comprises a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention and a second molecule of said binding pair comprises a binding region with which said binding partner interacts;

(b) means for contacting a sample suspected of containing or containing TSH or related ligands with said one or more pairs of binding molecules so as to permit said second molecule of said binding pair to interact with either (i) TSH or related ligands present in said sample, or (ii) said binding partner for the TSH receptor (typically a human monoclonal autoantibody); and

(c) means for monitoring the interaction of said second molecule of said binding pair with TSH or related ligands present in said sample, thereby providing an indication of the presence of TSH or related ligands in said sample.

A further application of a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention is its use to identify and provide new types of TSH receptor autoantibody binding sites. There is further provided by the present invention, therefore, a process of identifying one or more epitope regions of the TSH receptor, which process comprises contacting a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described with a full length TSH receptor, or one or more fragments thereof, so as to allow interaction of said binding partner for the TSH receptor with said full length TSH receptor, or said one or more fragments thereof, and identifying the amino acids of said full length TSH receptor, or said one or more fragments thereof, with which said binding partner interacts. Suitably, interaction of the binding partner with selected fragments of the TSH receptor and the full length TSH receptor, is analysed, so as to identify the amino acids of the TSH receptor with which the binding partner interacts.

Furthermore, the present invention allows for generation of antibodies to the regions of a monoclonal TSH receptor autoantibody according to the present invention which bind the TSH receptor. Such anti-idiotypic antibodies produced in this way could have potential as new ligands for assays of TSH receptor autoantibodies, TSH and related compounds. Also they may be effective agents in vivo for regulating the action of TSH receptor autoantibodies, TSH and related compounds. The present invention further provides, therefore, one or more anti-idiotypic antibodies generated

to binding regions of a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described.

Other methods of identifying and providing new types of autoantibody binding sites using monoclonal antibodies are well known. For example by antibody screening of phage-displayed random peptide libraries as described by JC Scott and GP Smith; "Searching for peptide ligands with an epitope library"; Science 1990; **249**(4967): 386-390 and MA Myers, JM Davies, JC Tong, J Whisstock, M Scealy, IR MacKay, MJ Rowley; "Conformational epitopes on the diabetes autoantigen GAD_{65} identified by peptide phage display and molecular modelling"; Journal of Immunology 2000; **165**: 3830-3838. Antibody screening of non-peptide compounds and libraries of non-peptide compounds can also be carried out.

New types of TSH receptor autoantibody binding sites identified and provided using these procedures may also be useful as new ligands in assays for TSH receptor autoantibodies, TSH and related compounds. Furthermore they may be effective agents in vivo for regulating the action of TSH receptor autoantibodies, TSH and related compounds.

A binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention substantially as hereinbefore described can also be usefully employed in therapy. There is, therefore, further provided by the present invention methods of treatment comprising administration of a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described, pharmaceutical compositions comprising a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described (together with one or more pharmaceutically acceptable carriers, diluents or excipients therefor), and use of a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described in the manufacture of a medicament or composition.

A binding partner for the TSH receptor, in particular a human monoclonal autoantibody to the TSH receptor derived from patients' lymphocytes according to the present invention, is a valuable reagent for understanding the pathogenesis of Graves'

disease and for developing new methods of measuring TSH receptor autoantibodies, for example as replacements for TSH in competitive binding assays substantially as hereinbefore described. Also, a stimulating binding partner according to the present invention has in vivo applications when tissue containing the TSH receptor (eg thyroid tissue or thyroid cancer tissue) requires stimulation. The present invention provides, therefore, a medicament or composition for use in stimulating thyroid tissue, and / or tissue containing the TSH receptor. In particular, a stimulating binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention can be employed in oncology, and in particular for use in the diagnosis, management and treatment of thyroid cancer. Alternatively, a binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention can be a powerful TSH antagonist (blocking antibody) and such a monoclonal TSH receptor autoantibody according to the present invention being a TSH receptor antagonist is valuable for in vivo applications when the activity of tissue containing the TSH receptor (eg thyroid tissue or thyroid cancer tissue) requires inactivation or to be made unresponsive to TSH, TSH autoantibodies or other stimulators.

One of the major advantages of a monoclonal antibody as provided by the present invention over TSH in such in vitro and / or in vivo applications is the relative ease with which such antibodies can be manipulated. For example, manipulation of the TSH receptor binding region of a monoclonal autoantibody according to the present invention so as to change the characteristics thereof, such as affinity and biological characteristics, including the degree of TSH agonist or antagonist activities. Also monoclonal antibodies according to the present invention have a much longer half life than TSH in vivo and this may have considerable advantages in in vivo applications. Furthermore, the half life of the antibodies can be manipulated, for example antibody Fab fragments have a much shorter half life than intact IgG.

Pharmaceutical compositions according to the present invention include those suitable for oral, parenteral and topical administration, although the most suitable route will generally depend upon the condition of a patient and the specific disease being treated. The precise amount of a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described to be

administered to a patient will be the responsibility of an attendant physician, although the dose employed will depend upon a number of factors, including the age and sex of the patient, the specific disease being treated and the route of administration substantially as described above.

There is further provided by the present invention a method of stimulating thyroid tissue, and / or tissue containing a TSH receptor, which method comprises administering to a patient in need of such stimulation a diagnostically or therapeutically effective amount of a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described.

The present invention also provides in combination, a binding partner for the TSH receptor (typically a human monoclonal autoantibody) substantially as hereinbefore described, together with one or more further agents capable of stimulating thyroid tissue, and / or tissue containing a TSH receptor, for simultaneous, separate or sequential use in stimulating thyroid tissue, and / or tissue containing a TSH receptor. Preferably the one or more further agents comprise recombinant human TSH and / or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments. Alternatively, the one or more further agents can act independently of binding to the TSH receptor.

A binding partner for the TSH receptor (typically a human monoclonal autoantibody) according to the present invention can also be employed as a replacement source for patient serum required to contain TSH receptor autoantibody or autoantibodies for use in commercial kits. Furthermore, a binding partner for the TSH receptor (typically a human monoclonal autoantibody) can be provided according to the present invention in a preparation required to comprise a defined concentration of TSH receptor autoantibody or autoantibodies, and in this way there can be provided a preparation with a defined activity, such as stimulatory activity, with respect to the TSH receptor. Optionally, such a preparation may further comprise one or more further human monoclonal autoantibodies, such as monoclonal autoantibodies to GAD, TPO or the like.

The following illustrative explanations are provided to facilitate understanding of certain terms used herein. The explanations are provided as a convenience and are not limitative of the invention

BINDING PARTNER FOR A TSH RECEPTOR, describes a molecule having a binding specificity for the TSH receptor. A binding partner as described herein may be naturally derived or wholly or partially synthetically produced. Such a binding partner has a domain or region which specifically binds to and is therefore complementary to one or more epitope regions of the TSH receptor. In particular, a binding partner as described herein can be a monoclonal antibody to the TSH receptor, and more particularly can be a human monoclonal autoantibody to the TSH receptor.

C DOMAIN denotes a region of relatively constant amino acid sequence in antibody molecules.

CDR denotes complementarity determining regions which are present on both heavy and light chains of antibody molecules and represent regions of most sequence variability. CDRs represent approximately 15 to 20% of variable domains and represent antigen binding sites of an antibody.

FR denotes framework regions and represent the remainder of the variable light domains and variable heavy domains not present in CDRs.

HC denotes part of a heavy chain of an antibody molecule comprising the heavy chain variable domain and the first domain of an IgG constant region.

HOST CELL is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous polynucleotide sequence.

IDENTITY, as known in the art, is the relationship between two or more polypeptide sequences, or two or more polynucleotide sequences, as determined by comparing the sequences.

LC denotes a light chain of an antibody molecule.

NIBSC 90/672 is an International Standard for thyroid stimulating antibody. The International Standard for thyroid stimulating activity consists of a batch of ampoules containing freeze dried plasma proteins from a single human patient with high TSH receptor autoantibodies. The preparation has been evaluated in an international collaborative study and shown to possess both thyroid stimulating and thyroid receptor binding activity. At the 46th meeting in 1995, the Expert Committee on Biological Standardization of WHO established the preparation coded 90/672 as the International Standard for thyroid stimulating antibody. Each ampoule contains freeze-dried residue of 1.0ml of a solution containing 0.02M phosphate buffer, dialysed human plasma proteins and 0.1 International Units (100 milli-International Units) per ampoule by definition.

STIMULATION OF A TSH RECEPTOR by a human monoclonal autoantibody as described herein denotes the ability thereof to bind to a TSH receptor and to thereby effect, for example, production of cyclic AMP as a result of such binding to the TSH receptor. Such stimulation is analogous to the responses seen on binding of TSH, or TSH receptor autoantibodies, to the TSH receptor and in this way a human monoclonal autoantibody as described herein essentially provides the same or similar binding responses as seen with TSH, or TSH receptor autoantibody, binding to a TSH receptor.

V DOMAIN denotes a region of highly variable amino acid sequence in antibody molecules.

V_HDOMAIN denotes variable regions or domains in heavy chains of antibody molecules.

V_LDOMAIN denotes variable regions or domains in light chains of antibody molecules.

The present invention will now be illustrated by the following Figures and Examples, which do not limit the scope of the invention in any way.

Examples

MATERIALS & METHODS

Lymphocyte isolation and cloning of human monoclonal TSH receptor autoantibodies

Blood was obtained from a patient with Graves' disease and Type 1 diabetes mellitus who had high levels of serum autoantibodies to the TSH receptor (TRAb). Ethical Committee approval was obtained for the studies. Peripheral blood lymphocytes were isolated on Ficoll–Paque (Amersham Biosciences; Chalfont St Giles, HP8 4SP, UK) from a 20mL blood sample and then infected with Epstein Barr virus (EBV) (European Collection of Cell Cultures – ECACC; Porton Down, SP4 0JG, UK) and cultured on mouse macrophage feeder layers as described before (N Hayakawa, LDKE Premawardhana, M Powell, M Masuda, C Arnold, J Sanders, M Evans, S Chen, JC Jaume, S Baekkeskov, B Rees Smith, J Furmaniak; "Isolation and characterization of human monoclonal autoantibodies to glutamic acid decarboxylase"; Autoimmunity 2002; **35**: 343-355). EBV immortalised B lymphocytes were then fused with the mouse/human hybrid cell line K6H6/B5 (WL Carroll, K Thilemans, J Dilley, R Levy; "Mouse x human heterohybridomas as fusion partners with human B cell tumors"; Journal of Immunological Methods 1986; **89**: 61-72) and cloned two times by limiting dilution at 5 cells/well and a final time at ½ cell/well to obtain a single colony (BJ Bolton, NK Spurr. "B-lymphocytes" In: RI Freshney, MG Freshney (eds). Culture of immortalized cells. Wiley-Liss, New York 1996; 283-297). The original wells and subsequent clones were screened for TSH receptor autoantibody by inhibition of ^{125}I-TSH binding to solubilised TSH receptor (see below). The single clones producing TSH receptor autoantibodies were grown up in tissue culture flasks.

Purification and labelling of monoclonal TSH receptor autoantibody preparations

IgGs were purified from tissue culture supernatants using affinity chromatography on Prosep A (Millipore UK Ltd.; Watford, WD18 8YH, UK) according to the manufacturer's instructions and purity assessed by SDS-polyacrylamide gel electrophoresis (PAGE).

Human heavy chain isotype was determined using a radial diffusion assay (The Binding Site; Birmingham, B29 6AT, UK). Human light chain isotype was determined using Western blotting with anti-human kappa chain and anti human lambda chain specific mouse monoclonal antibodies (Sigma-Aldrich Company Ltd; Gillingham, SP8 4XT, UK).

Mouse TSH receptor MAb IgGs were produced and purified as described before (Y Oda, J Sanders, M Evans, A Kiddie, A Munkley, C James, T Richards, J Wills, J Furmaniak, B Rees Smith; "Epitope analysis of the human thyrotropin (TSH) receptor using monoclonal antibodies"; Thyroid 2000; **10**: 1051-1059).

The purified IgG preparations were treated with mercuripapain (Sigma-Aldrich) at an enzyme/protein ratio of between 1:10 and 1:100 (depending on the particular monoclonal antibody) and passed through a Prosep A column to remove any intact IgG or Fc fragment from the Fab preparation (Y Oda, J Sanders, S Roberts, M Maruyama, R Kato, M Perez, VB Petersen, N Wedlock, J Furmaniak, B Rees Smith; "Binding characteristics of antibodies to the TSH receptor"; Journal of Molecular Endocrinology 1998; **20**: 233-244). Intact IgG was undetectable by SDS-PAGE in the Fab preparations. IgG and Fab preparations of the monoclonal antibodies were labelled with ^{125}I as described previously (Y Oda, J Sanders, S Roberts, M Maruyama, R Kato, M Perez, VB Petersen, N Wedlock, J Furmaniak, B Rees Smith; "Binding characteristics of antibodies to the TSH receptor"; Journal of Molecular Endocrinology; 1998; **20**: 233-244).

Patients

Sera from patients with Graves' disease of different disease duration were studied. The patients' sera studied showed inhibition of ^{125}I-labelled TSH binding to the TSH receptor (see below). In addition, sera from 2 patients with Addison's disease (A1 and A2) and high levels of autoantibodies to 21-OH (113 and 1970 units per mL, RSR kit) and 1 serum from a patient with type 1 diabetes mellitus (D1) with high levels of GAD_{65} (3700 units per mL; RSR kit) were studied. Informed consent for the study was obtained from the patients. Sera from healthy blood donors (purchased from

Golden West Biologicals, Vista, CA 92083, USA) were also studied. TRAb first international standard preparation (90/672) was obtained from the National Institute for Biological Standards and Control (NIBSC; Potters Bar, EN6 3QH, UK).

Inhibition of ^{125}I-TSH binding to the TSH receptor

TSH binding inhibition assays were carried out using TSH receptor coated tubes as described previously (J Sanders, Y Oda, S Roberts, A Kiddie, T Richards, J Bolton, V McGrath, S Walters, D Jaskolski, J Furmaniak, B Rees Smith; "The interaction of TSH receptor autoantibodies with ^{125}I-labeled TSH receptor"; Journal of Clinical Endocrinology and Metabolism 1999; **84**: 3797-3802) (reagents from RSR Ltd). Briefly, 100µL of sample (tissue culture supernatant, purified IgG or Fab fragment, patient serum or NIBSC 90/672 standards) were incubated in TSH receptor coated tubes at room temperature for 2 hours with gentle shaking. After aspiration, the tubes were washed twice with 1 mL of assay buffer (50 mmol/L NaCl, 10 mmol/L Tris-HCl pH 7.8, 0.1% Triton X-100) before addition of 100µL of ^{125}I-TSH (80,000 cpm) and incubation at room temperature for 1 hour with shaking. The tubes were then washed twice with 1mL of assay buffer, aspirated and counted in a gamma counter.

Inhibition of TSH binding was calculated as:-

$$100 \times 1 - \frac{\text{cpm TSH bound in the presence of test material}}{\text{cpm bound in the presence of control material}}$$

Control materials used were culture medium, a pool of healthy blood donor sera or as otherwise indicated.

Analysis of thyroid stimulating activities

The ability of monoclonal autoantibody preparations and patient sera to stimulate the production of cyclic AMP in CHO cells expressing hTSH receptor (approximately 50,000 receptors per cell) (Y Oda, J Sanders, S Roberts, M Maruyama, R Kato, M Perez, VB Petersen, N Wedlock, J Furmaniak, B Rees Smith; "Binding characteristics of antibodies to the TSH receptor"; Journal of Molecular Endocrinology 1998; **20**: 233-244) were carried out according to the method of R Latif, P Graves, TF Davies;

"Oligomerization of the human thyrotropin receptor"; Journal of Biological Chemistry 2001; **276**: 45217-45224. Briefly, CHO cells were seeded into 96 well plates (30,000 cells per well) and incubated for 24 hours in DMEM (Invitrogen Ltd; Paisley PA4 9RF, UK) containing 10% fetal calf serum. Culture was then continued in DMEM without fetal calf serum for a further 24 hours. The DMEM was then removed and test IgG, Fab and serum (diluted in NaCl free Hank's Buffered Salts solution containing 1g/L glucose, 20 mmol/L Hepes, 222 mmol/L sucrose, 15 g/L bovine serum albumin (BSA) and 0.5 mmol/L 3 isobutyl-1-methyl xanthine pH 7.4) added and incubated for 1 hour at 37°C. After removal of the test solutions, cells were lysed and assayed for cyclic AMP using a Biotrak enzyme immunoassay system from Amersham Biosciences; Chalfont St Giles, HP8 4SP, UK

Variable Region Gene Analysis

Total RNA was prepared from 1 x 10^7 cells of a TSH receptor autoantibody producing clone using the acid phenol guanidine method (P Chomczynski, N Sacchi; "Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction"; Analytical Biochemistry 1987; **162**: 156-159) and mRNA prepared using oligo dT magnetic beads (Dynal Biotech Ltd; Wirral, CH62 3QL, UK). RT-PCR reactions were performed using reagents from Invitrogen Ltd; Paisley PA4 9RF, UK. Sense strand oligonucleotide primers were designed using the sequences recommended by the Medical Research Council's V-base database (www.mrc-cpe.cam.ac.uk). Antisense primers specific for human IgG1 heavy chain and lambda light chain were based on constant region encoding DNA sequences. Both sense and antisense primers included additional 5' restriction endonuclease site sequences to facilitate cloning of PCR products. IgG1 heavy chain and lambda light chain RT-PCR reactions were performed using the complete panel of appropriate primers. All primers were synthesized by Invitrogen Ltd. The RT reaction took place at 50°C for 10 minutes followed immediately by 40 cycles of PCR (15 sec 94°C, 30 sec 55°C, 30 sec 72°C). RT-PCR products were cloned into pUC18 and DNA prepared using the Wizard kit from Promega UK Ltd; Southampton SO16 7NS, UK and sequenced by the Sanger-Coulson method (F Sanger, S Nicklen, AR Coulson; "DNA sequencing with chain terminating inhibitors"; Proceedings of the National Academy of Sciences

of the USA 1977; 74: 5463-5467). V region sequences were compared with available sequences of human Ig genes using Ig blast (www.ncbi.nlm.nih.gov/ igblast/igblast.cgi).

Immunoprecipitation Assay (IPA)

The cDNA encoding full length TSH receptor was placed downstream of the T7 promoter in pYES2 (Invitrogen) and used in an in vitro TnT system (Promega UK Ltd) to produce TSH receptor labelled with ^{35}S-methionine as previously described (L Prentice, J Sanders, M Perez, R Kato, J Sawicka, Y Oda, D Jaskolski, J Furmaniak, B Rees Smith; "Thyrotropin (TSH) receptor autoantibodies do not appear to bind to the TSH receptor produced in an in vitro transcription/translation system"; Journal of Clinical Endocrinology and Metabolism 1997; **82**: 1288-1292). Briefly 50µL ^{35}S-labelled TSH receptor (25 000 – 30 000 cpm) diluted in HSB (150 mmol/L Tris-HCL pH 8.3, 200 mmol/L NaCl and 10 mg/mL bovine serum albumin containing 1% Tween 20) were added to duplicate 50µL aliquots of diluted test sample and incubated for 2 hours at room temperature. Immune complexes were then precipitated by addition of protein A sepharose (Sigma-Aldrich) and counted in a scintillation counter.

TSH receptor preparations and western blotting

Full-length human TSH receptor was expressed in CHO-K1 cells, extracted with 1% Triton X-100 and purified by TSH receptor monoclonal antibody affinity chromatography as described previously (Y Oda, J Sanders, M Evans, A Kiddie, A Munkley, C James, T Richards, J Wills, J Furmaniak, B Rees Smith; "Epitope analysis of the human thyrotropin (TSH) receptor using monoclonal antibodies"; Thyroid 2000; **10**: 1051-1059).

The purified CHO cell produced TSH receptor was run on 9% SDS-PAGE gels, blotted onto nitrocellulose and reacted with test antibodies as described previously (Y Oda, J Sanders, M Evans, A Kiddie, A Munkley, C James, T Richards, J Wills, J

Furmaniak, B Rees Smith; "Epitope analysis of the human thyrotropin (TSH) receptor using monoclonal antibodies"; Thyroid 2000; **10**: 1051-1059).

Epitope analysis using TSH receptor peptides

Twenty six peptides each 25aa long covering the whole of the extracellular domain of the human TSH receptor were kindly provided by Dr J Morris (JC Morris, ER Bergert, DJ McCormick; "Structure-function studies of the human thyrotropin receptor. Inhibition of binding of labeled thyrotropin (TSH) by synthetic human TSH receptor peptides"; Journal of Biological Chemistry 1993; **268**: 10900-10905). A human 21-OH peptide (C1, SSSRVPYKDRARLPL) which binds to an M21-OH5 MAb (S Chen, J Sawicka, L Prentice, JF Sanders, H Tanaka, V Petersen, C Betterle, M Volpato, S Roberts, M Powell, B Rees Smith, J Furmaniak; "Analysis of autoantibody epitopes on steroid 21-hydroxylase using a panel of monoclonal antibodies"; Journal of Clinical Endocrinology and Metabolism 1998; **83**: 2977-2986) was used as a positive control and a human monoclonal antibody to GAD_{65} (N Hayakawa, LDKE Premawardhana, M Powell, M Masuda, C Arnold, J Sanders, M Evans, S Chen, JC Jaume, S Baekkeskov, B Rees Smith, J Furmaniak; "Isolation and characterization of human monoclonal autoantibodies to glutamic acid decarboxylase"; Autoimmunity 2002; **35**: 343-355) was used as a negative control. The peptide ELISA was carried out as described previously (Y. Oda, J Sanders, M Evans, A Kiddie, A Munkley, C James, T Richards, J Wills, J Furmaniak, B Rees Smith; "Epitope analysis of the human thyrotropin (TSH) receptor using monoclonal antibodies"; Thyroid 2000; **10**: 1051-1059).

Interaction of ^{125}I-labelled monoclonal TSH receptor autoantibody preparations with the TSH receptor

Test samples including patient sera (100µL) were incubated in TSH receptor coated tubes (RSR Ltd.) at room temperature for 2 hours with gentle shaking. After aspiration, the tubes were washed twice with 1mL of assay buffer before addition of 100µL of labelled autoantibody preparation (30,000 cpm) and incubation at room temperature for 1 hour with shaking. The tubes were then washed twice with 1mL of assay buffer, aspirated and counted in a gamma counter. Inhibition of ^{125}I-labelled

autoantibody binding was calculated using the formula as for inhibition of TSH binding (see above).

Scatchard analysis of monoclonal autoantibody binding to TSH receptor coated tubes

Unlabelled IgG or Fab in 50μL of assay buffer and 50μL of ^{125}I-labelled hMAb IgG or Fab (30,000 cpm in assay buffer) were incubated for 2 hours at room temperature with gentle shaking, washed twice with 1mL of assay buffer and counted in a gamma counter. The concentration of IgG or Fab bound vs bound/free was plotted (G Scatchard; "The attraction of proteins for small molecules and ions"; Annals of the New York Academy of Sciences 1949; 51: 660-672) to derive the association constants.

Binding of TSHR to tubes coated with monoclonal TSHR autoantibodies

Test samples including patient sera (100μL) and detergent solubilised TSHR (20μL) were incubated for 1 hour at room temperature. Duplicate 50μL aliquots of the incubation mixture were then added to plastic tubes (Nunc Maxisorp) which had been coated with monoclonal TSHR autoantibody Fab (200μL of 10μg/mL overnight at 4°C followed by washing and post coating). After incubation for 1 hour at room temperature with gentle shaking, the tubes were washed, 100μL (40,000 cpm) of ^{125}I-labelled TSHR C terminal monoclonal antibody 4E31 (J Sanders, Y Oda, A Kiddie, T Richards, J Bolton, V McGrath, S Walters, D Jaskolski, J Furmaniak, B Rees Smith; "The interaction of TSH receptor autoantibodies with ^{125}I-labelled TSH receptor"; Journal of Clinical Endocrinology and Metabolism 1999; **84**: 3797-3802) added and incubation continued for a further 1 hour with gentle shaking. Then tubes were then washed and counted for ^{125}I.

RESULTS

Lymphocytes (30 x 10^6) obtained from 20mL of patient's blood were plated out at 1 x 10^6 per well on a 48 well plate with 200μL of EBV supernatant on feeder layers of mouse macrophages. On day 11 post EBV infection the supernatants were monitored.

for inhibition of ^{125}I-TSH binding. One well was found to be positive for inhibition of binding, the levels of inhibition increasing to greater than 90% inhibition by day 16 and stayed at that level until day 24, after which time they decreased. The cultures were expanded and fused with K6H6/B5 cells on day 21, 23, 26 and 27 post EBV infection; in total 7 fusion experiments were carried out. Each fusion was plated across 3 x 96 well plates (ie 21 plates in total) and one well, stably producing antibodies with ^{125}I-TSH binding inhibiting activity was obtained. This was followed by 3 rounds of re-cloning to yield a single clone producing a human monoclonal antibody which inhibited labelled TSH binding to the TSH receptor. This human monoclonal TSH receptor autoantibody was designated hMAb TSHR 1 was of subclass IgG1 with a lambda light chain.

The ability of different concentrations of hMAb TSHR1 IgG and Fab to inhibit labelled TSH binding to the TSH receptor is shown in Figure 1. As can be seen in Figure 1 as little as 1ng/mL of three preparations inhibited TSH binding with more than 90% inhibition being obtained with 1000ng/mL. TSMAb TSHR1 IgG and Fab also stimulated cyclic AMP production in CHO cells transfected with the TSH receptor as shown in Figure 2. As little as 0.01ng/mL of hMAb TSHR1 Fab (2×10^{-13} molar) caused a 3 more than 3 fold stimulation of cyclic AMP. Approximately 3 fold stimulation of cyclic AMP was also observed with 0.2ng/mL of hMAb TSHR1 IgG (1.3×10^{-12} molar), 0.05ng/mL (1.8×10^{-12} molar) porcine TSH and 2.5ng/mL recombinant human TSH (9×10^{-11} molar). Comparison of the ability of the serum from the original lymphocyte donor (taken at the same time as the blood sample for lymphocyte isolation) to inhibit labelled TSH binding to the TSH receptor and not to stimulate cyclic AMP production in TSH receptor transfected CHO cells is shown in Figure 3. Inhibition of TSH binding could be detected with serum diluted 500x whereas stimulation of cyclic AMP could be detected with serum diluted 5000x. Similarly hMAb TSHR1 IgG gave detectable inhibition of TSH binding at about 1ng/mL and detectable stimulation of cyclic AMP production at about 0.1ng/mL ie hMAb TSHR1 IgG showed similar characteristics to the TSH receptor autoantibody activity in the donor patient serum.

^{125}I-labelled hMAb TSHR1 IgG bound to TSH receptor coated tubes and Scatchard analysis indicated an association constant of 5×10^{10} molar^{-1}. This binding was

inhibited by sera from patients with Graves' disease who had TSH receptor autoantibodies (detectable by inhibition of labelled TSH binding) (Table 1). ^{125}I-labelled hMAb TSHR1 Fab also bound to TSHR coated tubes (association constant by Scatchard analysis = 4.5 x 10^{10} molar^{-1}) and this binding was inhibited by TSHR autoantibody positive Graves' sera (Table 2). In addition, detergent solubilised preparations were able to bind to plastic tubes coated with hMAb TSHR1 and this binding could be inhibited by sera containing TSHR autoantibodies (Table 3).

hMAb TSHR1 IgG did not react with full length TSH receptor preparations on Western blot analysis nor did it react well with ^{35}S-labelled full length TSH receptor in the immunoprecipitation assay nor in the TSH receptor peptide ELISA. This lack of reactivity indicates that hMAb TSHR1 reacts with conformational rather than linear epitopes on the TSH receptor.

Sequence analysis of the genes coding for hMAb TSHR1 indicated that the heavy chain V region genes were of the VH5 family, the D gene of the D6-13 family and the J gene of the JH5 family. The heavy chain nucleotide and amino acid sequences are shown in Figures 4 and 5 respectively.

Comparison of the activities of hMAb TSHR1 IgG preparations and the international standard for TSHR autoantibodies in terms of inhibition of labelled TSH binding are shown in Table 4. This enabled a specific activity of hMAb TSHR1 IgG to be estimated as 138 units of NIBSC 90/672 per mg of protein when the assays were carried out in serum and 163 units per mg when the assays were carried out in assay buffer (mean of the 2 values = 150 units/mg). hMAb TSHR1 Fab preparations were 288 and 309 units per mg in serum and assay buffer respectively (mean of the 2 values = 300 units/mg).

Table 5 shows a similar analysis of the lymphocyte donor serum and the donor serum IgG. As can be seen the donor serum contains a mean of 0.38 units/mL of NIBSC 90/672 (0.36 and 0.4 in serum and assay buffer respectively) and the donor serum IgG has a mean specific activity of 0.059 units per mg of protein. These results are summarised in Table 6.

Activities of the various IgG and serum preparations in terms of stimulation of cyclic AMP in CHO cells transfected with the TSHR are also shown in Table 6. The specific activity of hMAb TSHR1 IgG was 180 units of NIBSC 90/672 per mg of protein and the Fab preparation 700 units per mg of protein. The lymphocyte donor serum contained 1.8 units/mL and the donor serum IgG had a specific activity of 0.33 units per mg as measured by stimulation of cyclic AMP.

CONCLUSIONS

(a) We have produced a human monoclonal autoantibody to the TSH receptor which has similar properties to the TSH receptor autoantibody in the donor patient's serum.

(b) The monoclonal antibody IgG and Fab preparations are powerful thyroid stimulators and effective inhibitors of labelled TSH binding to the TSH receptor.

(c) Binding of labelled MAb IgG and Fab preparations to the TSH receptor is inhibited by TSH receptor autoantibody positive sera from patients with Graves' disease but not by healthy blood donor sera or sera from patients with other autoimmune diseases.

(d) TSH receptor autoantibodies which act as TSH antagonists as well as TSH receptor autoantibodies which act as TSH agonists inhibit labelled hMAb TSHR1 binding to the TSH receptor.

(e) hMAb TSHR1 preparations coated onto plastic tubes bind TSHR and this binding is inhibited by TSHR autoantibodies in different patient sera.

(f) These results indicate that hMAb TSHR1 and/or its derivatives can be used as a replacement for TSH in

(i) assays for TSH receptor autoantibodies, TSH and related ligands

(ii) various in vivo applications involving provision of TSH agonist or TSH antagonist activities.

(iii) identification and provision of new types of TSH receptor autoantibody binding sites.

Sequence Listings

SEQ ID NO. 1

SGAEVKKPGESLKISCRGSGYRFTSYWINWVRHVPGKGLEWMGRIDPTDSYT
NYSPSFKGHVTVSADKSINTAYLQWSSLKASDTGMYYCARLEPGYSSTWSVN
WGQGTLVTVSS

SEQ ID NO. 2

SYWIN

SEQ ID NO. 3

RIDPTDSYTNYSPSFKG

SEQ ID NO. 4

LEPGYSSTWSVN

SEQ ID NO. 5

SGAEVKKPGESLKISCRGSGYRFTSYWINWVRHVPGKGLEWMGRIDPTDSYT
NYSPSFKGHVTVSADKSINTAYLQWSSLKASDTGMYYCARLEPGYSSTWSVN
WGQGTLVTVSSASTKGPSVFP

SEQ ID NO. 6

tctggagcagaggtgaaaaagcccggggagtctctgaagatcctgtaggggttctggatacaggtttaccagctactgg

atcaactgggtgcgccacgtgcccgggaaaggcctagagtggatgggcaggattgatcctactgactcttataccaactac

agtccatccttcaaaggccacgtcaccgtctcagctgacaagtccatcaacactgcctacctgcagtggagcagcctgaag

gcctcggacaccggcatgtattactgtgcgaggctcgaaccgggctatagcagcacctggtccgtaaattggggccaggg

aaccctggtcaccgtctcctca

SEQ ID NO. 7

agctactggatcaac

SEQ ID NO. 8

aggattgatcctactgactcttataccaactacagtccatccttcaaaggc

SEQ ID NO. 9

ctcgaaccgggctatagcagcacctggtccgtaaat

SEQ ID NO. 10

tctggagcagaggtgaaaaagcccggggagtctctgaagatctcctgtaggggttctggatacaggtttaccagctactgg

atcaactgggtgcgccacgtgcccgggaaaggcctagagtggatgggcaggattgatcctactgactcttataccaactac

agtccatccttcaaaggccacgtcaccgtctcagctgacaagtccatcaacactgcctacctgcagtggagcagcctgaag

gcctcggacaccggcatgtattactgtgcgaggctcgaaccgggctatagcagcacctggtccgtaaattggggccaggg

aaccctggtcaccgtctcctcagcctccaccaagggcccatcggtcttccccc

Table 1 Effect of patient sera on ^{125}I-labelled hMAb TSHR1 binding to the TSHR

Test material	inhibition of labelled hMAb TSHR1 binding	inhibition of TSH binding	Test material	inhibition of labelled hMAb TSHR1 binding	inhibition of TSH binding
G1	62	80	N1	3.1	7.7
G2	91	93	N2	2.4	2.6
G3	91	76	N3	-1.0	4.5
G4	94	92	N4	-11	6.5
G5	93	94	N5	1.7	5.0
G6	76	85	N6	2.8	1.7
G7	87	90	N7	5.2	-0.8
G8	65	45	N8	3.5	0.2
G9	88	90	N9	2.8	-0.6
G10 /10	83	59	N10	4.5	2.2
G10 /20	69	43	D1	-4.8	2.2
G10 /40	56	29	A1	-3.1	1.3
G10 /80	42	19	A2	-3.5	-3.0
G11 /10	75	73			
G11 /20	59	54			
G11 /40	39	33			
G11 /80	22	18			

G1-G11 are sera from patients with a history of Graves' disease.

G9 serum has high levels of TSH blocking (ie TSH antagonist activity).

G10 and G11 have high levels of thyroid stimulating activity.

G10 is the lymphocyte donor serum.

/10, /20 etc indicate dilution factor in a pool of healthy blood donor sera.

N1-N10 are sera from healthy blood donors.

D1 is from a patient with type 1 diabetes mellitus (positive for autoantibodies to glutamic acid decarboxylase).

A1 and A2 are from patients with Addison's disease (positive for steroid 21-hydroxylase autoantibodies).

In the presence of the pool of healthy blood donor sera about 25% of ^{125}I-labelled MAb IgG bound to the TSHR coated tubes.

Table 2 Effect of patient sera on ^{125}I-labelled hMAb TSHR1 Fab binding to the TSHR

Test material	inhibition of labelled Fab binding	inhibition of TSH binding
90/672 diluted in a pool of healthy blood donor serum		
to 1 U/L	17	13
to 2 U/L	27	24
to 4 U/L	47	44
to 8 U/L	61	65
Healthy blood donor serum A	-3	<10
Healthy blood donor serum B	3	<10
Healthy blood donor serum C	4	<10
Healthy blood donor serum D	-4	<10
Healthy blood donor serum E	0	<10
Graves' serum F	64	78
Graves' serum G	42	54
Graves' serum H	49	69
Graves' serum I	24	36
Graves' serum J	76	88

Table 3 Binding of TSHR to plastic tubes coated with hMAb TSHR 1 Fab and inhibition of TSHR binding by sera containing TSHR autoantibodies

Test material	cpm bound[1]
Healthy blood donor serum A	8406
Healthy blood donor serum B	8430
TSHR autoantibody positive serum 1	1527
TSHR autoantibody positive serum 2	1131
TSHR autoantibody positive serum 3	1199

[1] TSHR binding was detected using a ^{125}I-labelled mouse monoclonal antibody to the TSHR C terminus; total cpm = 39,000 per tube.

Table 4 **Inhibition of TSH binding by WHO reference preparation NIBSC 90/672 and by hMAb TSHR1 IgG and Fab preparations**

Sample	Samples diluted in serum[1]				Samples diluted in assay buffer			
	% inhibition	units/L	units/mg	mean units/mg	% inhibition	units/L	units/mg	mean units/mg
NIBSC 90/672								
0.125 units/L					2			
0.25 units/L					4			
0.5 units/L					11			
1.0 units/L	15				19			
2.0 units/L	28				38			
4.0 units/L	48				64			
8.0 units/L	69				83			
40.0 units/L	95				94			
hMAb TSHR1 IgG								
0 ng/mL	1				0			
0.3 ng/mL	1				2			
1 ng/mL	3				3			
3 ng/mL	7				10	0.46		
10 ng/mL	21	1.48	148		33	1.73	173	
30 ng/mL	46	3.9	130	138	70	4.8	160	163
100 ng/mL	81	13.5	135		92	15.6	156	
300 ng/mL	92				95	>40		
hMAb TSHR1 Fab								
0.3 ng/mL	5				-2			
1 ng/mL	5				1			
3 ng/mL	16	1.05	351		16	0.8	265	
10 ng/mL	36	2.77	277		52	2.9	291	309
30 ng/mL	69	8.0	267	288	86	9.6	372	
100 ng/mL	89	23.7	237		92	16.9		
300 ng/mL	93				94			
2G4 IgG[2]								
0.3 ng/mL	2				-3			
3 ng/mL	1				-6			
30 ng/mL	0				-5			
300 ng/mL	3				-4			
2G4 Fab[2]								
0.3 ng/mL	4				-5			
3 ng/mL	4				-6			
30 ng/mL	1				-5			
300 ng/mL	2				-6			

[1] Pool of healthy blood donor serum, 14.9% of total cpm bound to the TSHR in the presence of this serum pool only. 14.7% of total cpm bound to the TSHR in the presence of buffer only.

[2] 2G4 is a human monoclonal autoantibody to thyroid peroxidase.

Table 5 **Inhibition of TSH binding by lymphocyte donor serum and donor serum IgG**

Sample	Samples diluted in serum[1]				Samples diluted in assay buffer			
	% inhibition	units/L[2]	units/mg or (units/mL in undiluted serum)	mean units/mg or (units/mL)	% inhibition	units/L[2]	units/mg or (units/mL in undiluted serum)	mean units/mg or (units/mL)
Donor serum								
diluted 1000x	6				10			
diluted 300x	18	1.2	(0.36)		28	1.3	(0.39)	
diluted 100x	42	3.2	(0.32)	(0.36)	62	3.9	(0.39)	(0.40)
diluted 30x	78	11.3	(0.39)		91	13.5	(0.41)	
diluted 10x	93	34			95	>40		
Donor serum IgG								
0 mg/mL	0	0			0			
0.01 mg/mL	7				19	0.87		
0.03 mg/mL	23	1.6	0.053		37	1.9	0.063	
0.1 mg/mL	57	5.1	0.051	0.054	78	6.4	0.064	0.063
0.3 mg/mL	85	17	0.057		93	19	0.063	
1 mg/mL	96	43			96	>40		
Healthy blood donor pool serum								
diluted 1000x	0				3			
diluted 100x	1				4			
diluted 10x	1				11			
Healthy blood donor pool serum IgG								
0.01 mg/mL	2				2			
0.1 mg/mL	1				5			
1 mg/mL	3				7			

[1] Pool of healthy blood donor serum, 14.7% of total cpm bound to the TSHR in the presence of this serum pool only. 16.3% of total cpm bound to the TSHR in the presence of buffer only.

[2] Units shown are NIBSC 90/672 international TSHR autoantibody reference preparation.

Table 6 **Specific activities of hMAb TSHR1 and lymphocyte donor serum and IgG preparations**

	Inhibition of TSH binding assay		Stimulation of cyclic AMP assay	
Preparation	**Units/mg[1,2]**	**Units/nmole[1,2]**	**Units/mg[1]**	**Units/nmole[1]**
hMAb TSHR1 IgG	150	22	180	26
hMAb TSHR1 Fab	300	15	700	35
Donor serum IgG	0.059	0.009	0.33	0.048
Donor serum units/mL	0.38		1.8	

[1] Units shown are NIBSC 90/672.
[2] Values are a mean of results obtained in serum and in assay buffer (see Tables 4 and 5)

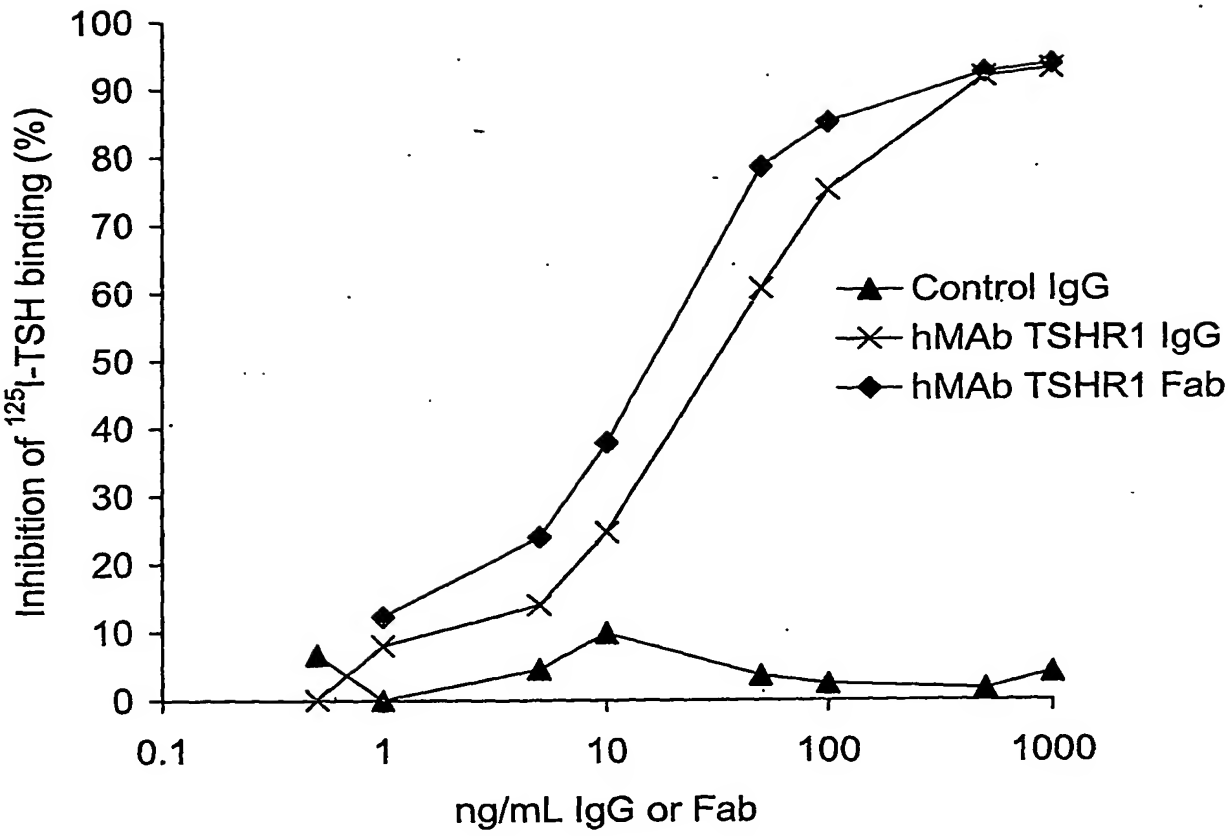

Figure 1 Inhibition of labelled TSH binding to TSHR coated tubes by hMAb TSHR1 IgG and Fab. The control IgG was a human monoclonal autoantibody to GAD_{65}.

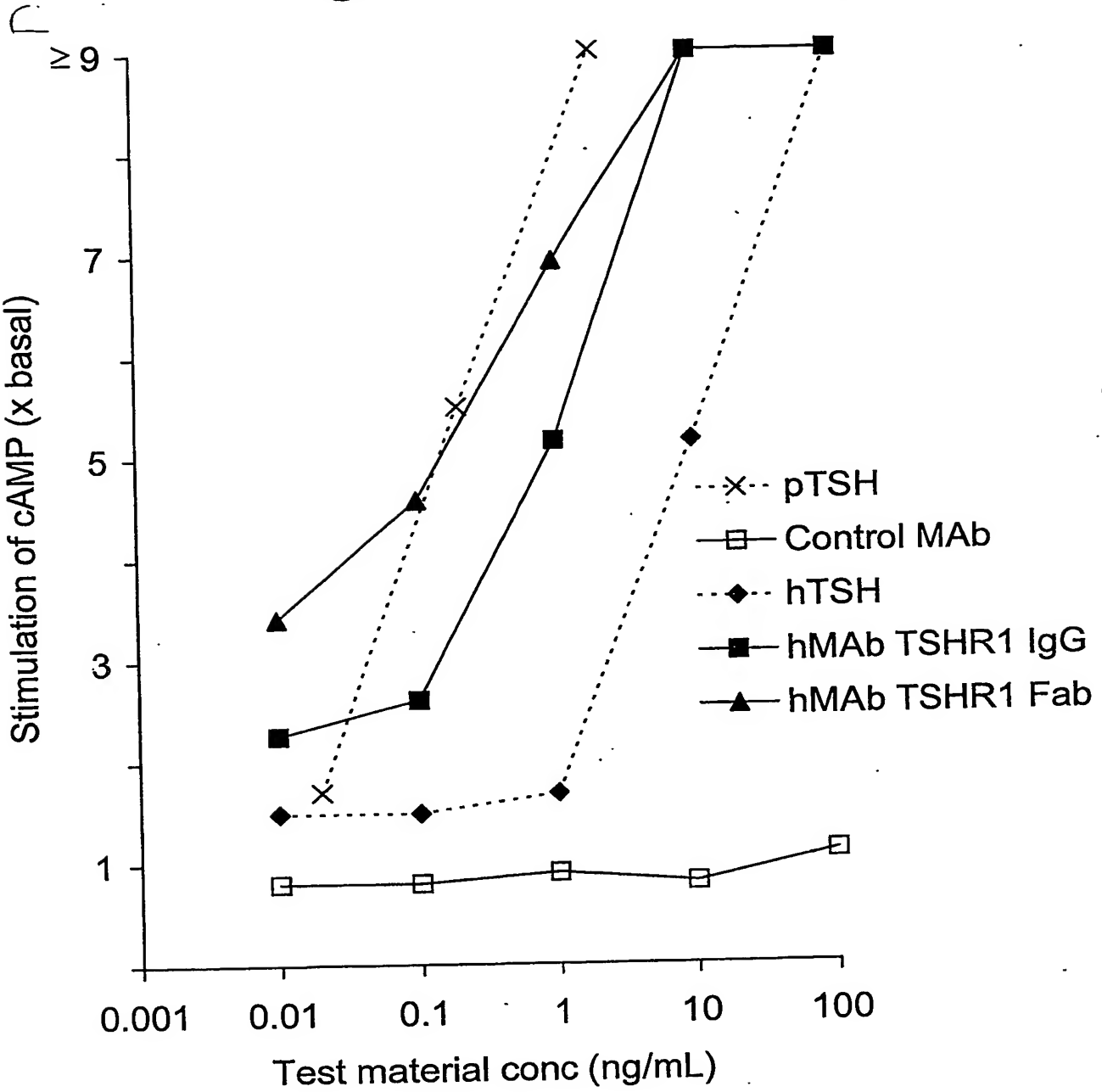

Figure 2 Thyroid stimulating activities of hMAb TSHR1 IgG and Fab, porcine TSH (70 units/mg; pTSH), recombinant human TSH (6.7 units/mg; hTSH) and a control monoclonal antibody (MAb: a human monoclonal autoantibody to thyroid peroxidase).

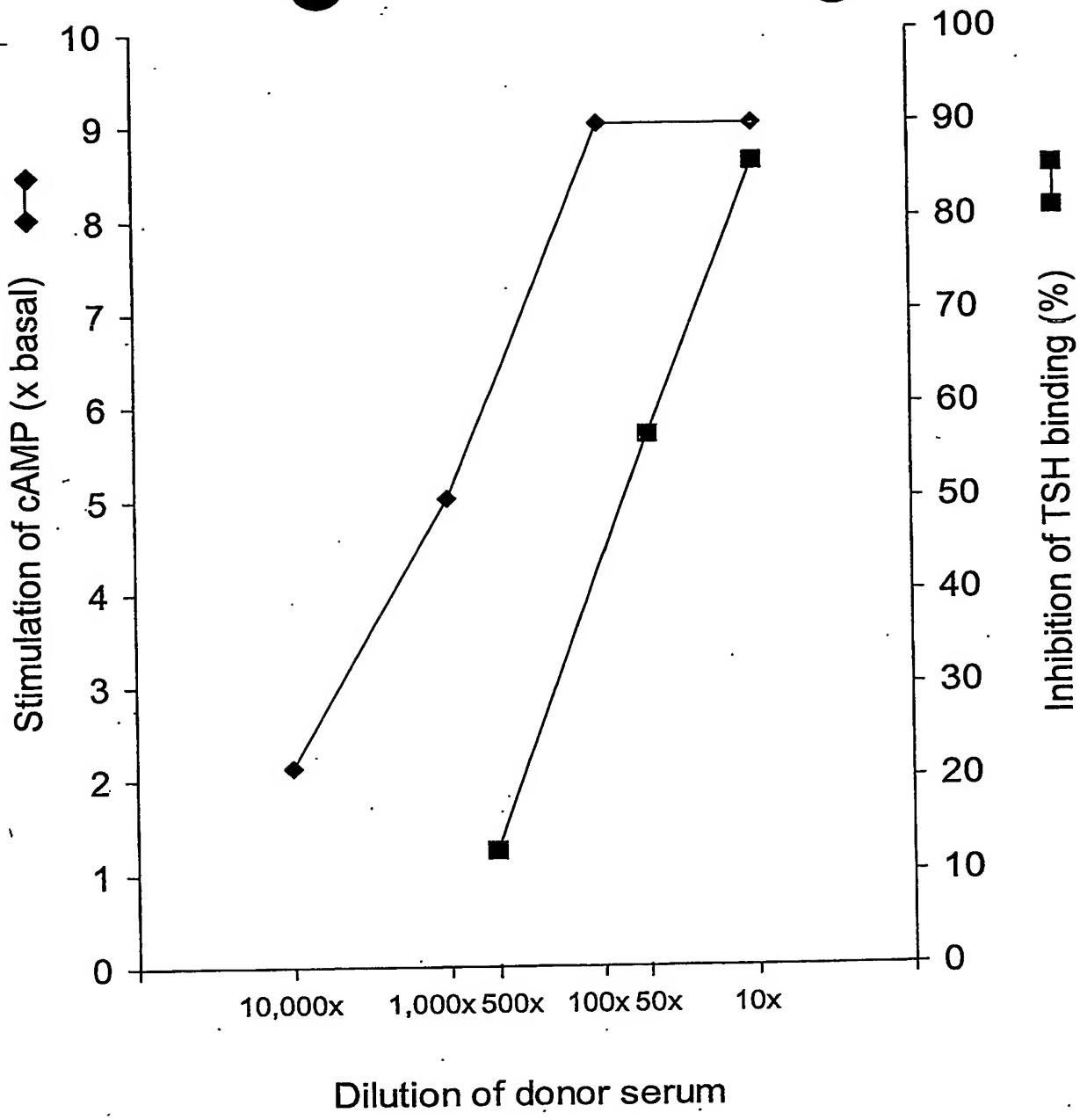

Figure 3 Effect of lymphocyte donor serum on inhibition of TSH binding to the TSHR and on stimulation of cyclic AMP in TSHR transfected CHO cells. In the case of the binding inhibition assay the serum was diluted in a pool of healthy blood donor sera. For the stimulation assay, the serum was diluted in NaCl free Hanks Buffered Salt Solution. Healthy blood donor sera (n = 3) gave responses ranging from 1.1 – 1.3 x basal.

Figure 4 hMAb TSHR1 Heavy Chain V, D and J region nucleotide sequence

caaatgcagctggtgcagtctggagcagaggtgaaaaagcccggggagtc

tctgaaagatctcctgtaggggttctggatacaggtttaccagctactgga

tcaactgggtgcgccacgtgcccgggaaaggcctagagtggatgggcagg

attgatcctactgactcttataccaactacagtccatccttcaaaggcca

cgtcaccgtctcagctgacaagtccatcaacactgcctacctgcagtgga

gcagcctgaaggcctcggacaccggcatgtattactgtgcgaggctcgaa

ccgggctatagcagcacctggtccgtaaattggggccagggaaccctggt

caccgtctcctcagcctccaccaagggcccatcggtcttccccc

caaatgcagctggtgcagtctggagcagaggtgaaaaagcccggggagtc 50

PCR primer

tctgaaagatctcctgtaggggttctggatacaggtttaccagctactgga 100

CDR I

tcaactgggtgcgccacgtgcccgggaaaggcctagagtggatgggcagg 150

CDR II

attgatcctactgactcttataccaactacagtccatccttcaaaggcca 200

cgtcaccgtctcagctgacaagtccatcaacactgcctacctgcagtgga 250

gcagcctgaaggcctcggacaccggcatgtattactgtgcgaggctcgaa 300

CDR III

ccgggctatagcagcacctggtccgtaaattggggccagggaaccctggt 350

constant region

caccgtctcctca**gcctccaccaagggcccatcggtcttccccc** 394

Figure 5 hMAb TSHR1 Heavy Chain V, D and J region amino acid sequence

QMQLVQSGAEVKKPGESLKISCRGSGYRFTSYWINWVRHVPGKGLEWMGR

IDPTDSYTNYSPSFKGHVTVSADKSINTAYLQWSSLKASDTGMYYCARLE

PGYSSTWSVNWGQGTLVTVSSASTKGPSVFP

QMQLVQSGAEVKKPGESLKISCRGSGYRFTSYWINWVRHVPGKGLEWMGR 50

PCR primer

CDR I

IDPTDSYTNYSPSFKGHVTVSADKSINTAYLQWSSLKASDTGMYYCARLE 100

CDR II

PGYSSTWSVNWGQGTLVTVSS**ASTKGPSVFP** 131

constant region

CDR III